# Net flux of nonesterified fatty acids, cholesterol, triacylglycerol, and glycerol across the portal-drained viscera and liver of pregnant ewes

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# Net flux of nonesterified fatty acids, cholesterol, triacylglycerol, and glycerol across the portal-drained viscera and liver of pregnant ewes[1,2]

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**ABSTRACT:** The objective of this study was to determine the pattern of nutrient flux across portal-drained viscera (PDV) and liver in ewes with varying numbers of fetuses. Catheters were placed in the hepatic portal vein, a branch of the hepatic vein, a mesenteric vein, and the abdominal aorta of each ewe. Plasma flow and net cholesterol, nonesterified fatty acids (NEFA), and glycerol release across the PDV and liver were determined prior to exposure to rams. Ewes were subsequently mated. Two ewes were not pregnant, six ewes gave birth to singles, and 11 ewes gave birth to twins. Additional measurements were taken 103, 82, 61, 39, 19, and 6 d before parturition. There was a net PDV uptake of nonesterified cholesterol in the nonpregnant ewes and a net release in the ewes with singles and twins. Net nonesterified cholesterol hepatic release did not differ with days from parturition ($P$ = .77). There was a net hepatic release of nonesterified cholesterol in the ewes with twins and a net hepatic uptake in the ewes with singles and in nonpregnant ewes ($P$ = .03). There was a net PDV release of NEFA; however, it did not differ with litter size ($P$ = .59) or days from parturition ($P$ = .63). Hepatic NEFA uptake increased with litter size ($P$ = .03) and increased as gestation progressed ($P$ = .006). There was an interaction ($P$ = .04) between litter size and days from parturition for net PDV glycerol release. Net PDV glycerol release in the nonpregnant ewes decreased over time, but release in pregnant ewes tended to increase over time. Hepatic glycerol uptake increased with litter size and increased as gestation progressed. There was a net PDV uptake of triacylglycerol, but it did not differ with litter size ($P$ = .11) or with days from parturition ($P$ = .06). There was a net hepatic release of triacylglycerol, but it did not differ with litter size ($P$ = .59) or with days from parturition ($P$ = .67). Liver utilization of glycerol and NEFA as substrates for metabolism increases as pregnancy progresses. In the nonpregnant ewe, the combined contribution of glycerol and NEFA carbon accounted for 10% of the carbon taken up by the liver, and in ewes pregnant with twins, the combined contribution accounted for 42% of the carbon uptake 19 d before parturition. In conclusion, these data demonstrate NEFA are an important metabolite when determining carbon balance across the liver and their relative contribution to carbon balance increases as pregnancy progresses.

Key Words: Ruminants, Sheep, Metabolism, Plasma Flow

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## Introduction

Nutrient flux studies have quantified specific nutrient requirements of the gravid uterus at different stages of pregnancy (Battaglia and Meschia, 1981; Bell et al., 1986). As metabolites required by the gravid uterus change, the intermediary metabolism of the ewe adapts to meet those needs. The metabolism of the maternal liver changes during pregnancy (van der Walt et al., 1983; Freetly and Ferrell, 1998) in a manner that supports the metabolic demands of the gravid uterus. Net flux studies across the portal-drained viscera (**PDV**) and liver provide an opportunity to quantify the change in intermediary metabolism and the coordination between tissues as pregnancy progresses. Freetly and Ferrell (1998) indicated that the net hepatic uptake of the metabolites accounted for 64 to 73% of the carbon released by the liver 19 d before parturition. Therefore, it was hypothesized that quantitatively important sources of carbon were not accounted for. In order to describe hepatic metabolism during pregnancy adequately, it is important to define the sources of carbon that have not been accounted for. In a similar analysis

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[2]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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of carbon flux across the liver of lactating cows, Freetly et al. (1993) proposed lipids as the potential sources of unaccounted carbon released by the liver. It was the objective of this study to determine the pattern of net uptake of metabolites associated with lipids across the PDV and liver in ewes with varying numbers of fetuses. Because fetal number and stage of pregnancy affect aerobic metabolism across the liver, it was postulated the fetal number would alter lipid metabolism and its potential contribution to carbon flux.

## Materials and Methods

### *Animal Management*

Details of animal management and sampling protocols have been previously presented (Freetly and Ferrell, 1997). Briefly, 19 multiparous, polled, Dorset ewes were individually penned. Sheep were provided water and a pelleted diet for ad libitum consumption (57% dehydrated alfalfa, 28% corn cobs, and 15% corn, DM basis; CP 11%). Ewes were fed daily at 1300 and feed refusal from the previous day was determined at feeding. A digestion study in lambs determined that the diet had an apparent digestible energy concentration of 1.90 Mcal/kg DM (Freetly and Ferrell, 1997). Catheters were surgically placed in the portal vein, a branch of the hepatic vein, a mesenteric vein, and the abdominal aorta. Ewes were bred 54 d after surgery. Experimental procedures were conducted in accordance with the Meat Animal Research Center Animal Care Guidelines and the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Consortium, 1988).

### *Sampling Protocol*

Each ewe was transferred to a metabolism crate 47 d after surgery (151 d before parturition). A priming dose (15 mL) of *p*-aminohippuric acid (.15 *M*; **PAH**) was infused via the mesenteric vein catheter, followed by a constant infusion (.8 mL/min). Sixty minutes following the priming dose, blood samples were drawn into syringes containing 15 IU heparin (10 mL) or 11 mg EDTA (5 mL) from the aortic, portal venous, and hepatic venous catheters. Samples were collected at 30-min intervals for a total of seven sets of samples per period (aortic, portal venous, and hepatic venous). Additional samples were collected as above 103, 82, 61, 39, 19, and 6 d before parturition. Plasma was obtained from the EDTA-treated samples and analyzed for cholesterol, NEFA, and glycerol.

Plasma flow was calculated using an indicator-dilution technique previously described (Katz and Bergman, 1969b). Net release of metabolites was calculated by multiplying plasma flow by the concentration difference in the vessels (Katz and Bergman, 1969b). In the tables, a negative release rate represents a net uptake by the tissue.

An automated assay for use on a Technicon Autoanalyzer II was developed for the measure of total cholesterol. The premise of the assay is that cholesterol esterase is used to convert cholesterol esters to cholesterol and free fatty acids. Cholesterol oxidase is then used to convert cholesterol and oxygen to cholesterol-4-en-3-one and hydrogen peroxide. In the presence of peroxidase the hydrogen peroxide reacts with 3-methyl-2-benzothiazolinone hydrazone (**MBTH**) and N,N-dimethylaniline (**DMA**) to produce indamine dye.

The cholesterol esterase/oxidase working solution contained 100 U each of cholesterol esterase (EC 3.1.1.13) and cholesterol oxidase (EC 1.1.3.6) in 1 L of phosphate buffer plus 1.5 mL of Triton X-100 per liter of phosphate buffer. The phosphate buffer contained $Na_2HPO_4$ (184.3 m*M*), $KH_2PO_4$ (77.9 m*M*), and $MgCl_2$ (4.9 m*M*), and pH was adjusted to 7.45 with NaOH and HCl. The peroxidase working solution contained 4,500 U of Type I horseradish peroxidase (EC 1.11.1.7) in 1 L of deionized water.

The working solution for MBTH/DMA was prepared from stock solutions of MBTH and DMA. Stock MBTH contained MBTH (4.64 m*M*) and HCl (.1 *N*). Stock DMA contained DMA (79.21 m*M*) and HCl (.1 *N*). The MBTH/DMA working solution was made by combining 20 mL stock MBTH, 30 mL stock DMA, 50 mL acetic acid (10 *N*), and 900 mL distilled water. The MBTH/DMA working solution contained MBTH (.093 m*M*), DMA (2.376 m*M*), acetic acid (500 m*N*), and HCl (5 m*N*).

The manifold was arranged such that the cholesterol esterase/oxidase working solution was introduced at a rate of 1 mL/min, followed by air at a rate of .32 mL/min, followed by sample at .1 mL/min. The solution was then passed through a five-turn mixing coil and through two heating baths arranged in series. The first bath was equipped with a 5.37-mL coil and the second with a 7.70-mL coil. Both baths were set to 37°C. The solution was passed through a 10-turn mixing coil, and the peroxidase solution was added at the rate of .23 mL/min. The solution was then passed through two 10-turn mixing coils set in series before it entered the colorimeter. The colorimeter was equipped with a 1 mL/min line on a 1.5-mL, 15-mm flowcell and had a pair of 600-nm filters. The sampler was set for 40 samples/hour with a 2:1 sample:wash ratio. Nonesterified cholesterol was determined with the same assay procedure, except that the cholesterol esterase was omitted from the cholesterol esterase/oxidase working solution.

The assay was linear up to .26 m*M*. The intraassay CV for total cholesterol was .6%, and for nonesterified cholesterol it was 2.2%. The interassay CV for total cholesterol was 2.1%, and for nonesterified cholesterol it was 3.9%. Plasma samples were diluted 1:10 with water before analysis.

Plasma samples for triacylglycerol/glycerol and NEFA were diluted 1:3 with a phosphate buffer. The buffer contained $Na_2HPO_4$ (38.5 m*M*) and $KH_2PO_4$ (25.3 m*M*), and pH was adjusted to 6.9 with NaOH.

Plasma triacylglycerol and glycerol were determined using Sigma procedure no. 337 modified for use with a 96-well plate. The procedure uses lipoprotein lipase (EC 3.1.1.3) to convert triacylglycerol to glycerol and fatty acids. In the presence of glycerol kinase (EC 2.7.1.30) and ATP, glycerol is converted to glycerol-1-phosphate. In the presence of glycerol phosphate oxidase (EC 1.1.3.21), glycerol-1-phosphate is converted to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase (EC 1.11.1.7), hydrogen peroxide catalyzes the coupling of 4-aminoantipyrine and sodium N-ethyl-N(3-sulfopropyl)m-anisidine to form a quinoneimine dye.

Polystyrene plates with 365-μL wells were used for the assay. Plasma glycerol concentrations were determined by adding 25 μL of buffered plasma to each well plus 200 μL of reagent A [ATP, magnesium salt, 4-aminoantipyrine, sodium-N-ethyl-N(3-sulfopropyl)m-anisidine, glycerol kinase, glycerol phosphate oxidase, and peroxidase]. The plate was mixed and incubated for 10 min at 25°C, and absorption was determined at 550 nm. Total glycerol concentration was determined by adding 50 μL reagent B (lipoprotein lipase), mixing, and incubating the plate at 37°C for 30 min. Absorption at 550 nm was then determined. Triglyceride concentrations were determined as the difference in concentration between total glycerol and plasma glycerol (nonesterified). The intraassay CV for total glycerol was 4.5%, and for nonesterified glycerol it was 7.5%. The interassay CV for total glycerol was 7.7%, and for nonesterified glycerol it was 12.4%.

Nonesterified fatty acids were measured using a modification of the technique reported by Johnson and Peters (1993), which is itself a modification of a commercially prepared kit (NEFA-C, Wako Chemical USA, Richmond, VA). Reagents were diluted as described by Johnson and Peters (1993). Polystyrene plates with 365-μL wells were used for the assay. Plasma NEFA concentrations were determined by adding 50 μL of buffered plasma and 75 μL of reagent A to each well. The plate was mixed and incubated for 30 min at 37°C. Reagent B (125 μL) was added at the end of the incubation, and the plate was mixed and allowed to incubate 30 min at 25°C. The absorption at 550 nm was then determined. The assay was linear for concentrations between .15 and .45 m*M*. Plasma samples that exceeded .45 m*M* were diluted 1:6 with phosphate buffer. The intraassay CV was 2.9%, and the interassay CV was 9.4%.

Data were analyzed as a split-plot in time. The model included litter size and period as fixed effects. The model was litter size, ewe nested within litter size, period, and litter size × period. Litter size was tested with ewe nested within litter size as the source of error. Differences between least squares means were tested with a protected *t*-test. Means and standard errors are presented in the text, tables, and figures. For discussion, responses with probabilities less than .05 are considered to be different. Statistical analyses were conducted with the GLM procedure in SAS release 6.12 (Cary, NC).

## Results

Pregnancy was confirmed 35 d following mating with ultrasound. Two ewes did not give birth to lambs (**Nonpregnant**). Six ewes gave birth to a single (**Single**), and 11 ewes gave birth to twins (**Twin**). Over the course of the study some of the ewes had catheters that were not patent. One ewe in the Single group had a catheter in the hepatic vein that failed to function during Periods 5 and 7. In the Twin group, one ewe was removed from the study after Period 3, and a second ewe was removed after Period 4 due to failure of the abdominal aortal catheter. One ewe in the Twin group lambed before Period 7 and therefore was not sampled during Period 7. Some of the catheters became difficult to sample during the course of the study, resulting in a limited amount of blood at times. In cases in which blood was limited, not all chemistries were conducted. Feed intake tended to decrease during pregnancy, and BW increased (Freetly and Ferrell, 1997).

### *Cholesterol*

Arterial (.296 ± .004 m*M*), portal venous (.298 ± .005 m*M*), and hepatic venous (.297 ± .005 m*M*) concentrations of nonesterified cholesterol did not differ with days from parturition ($P \geq .19$) or litter size ($P \geq .73$). There was a net PDV uptake of nonesterified cholesterol in the nonpregnant ewes (.776 ± .280 mmol/h) and a net release in the ewes with singles (.863 ± .333 mmol/h). Net PDV uptake of nonesterified cholesterol in ewes with twins did not differ from zero (.112 ± .220 mmol/h). Net hepatic release did not differ with days from parturition ($P = .77$) but did differ with litter size ($P = .03$). There was a net release of nonesterified cholesterol in the ewes with twins (.386 ± .176 mmol/h) and a net uptake in the ewes with singles (.644 ± .247 mmol/h) and in nonpregnant ewes (.303 ± .113 mmol/h).

Arterial concentration of esterified cholesterol (.904 ± .015 m*M*) did not differ with litter size ($P = .55$) but did differ with days from parturition ($P = .01$). The difference in days from parturition resulted from the concentrations at 151 d before parturition (.781 ± .030 m*M*) being lower than the concentration at 103 d before parturition (.907 ± .028 m*M*). Portal venous concentration of esterified cholesterol (.897 ± .015 m*M*) did not differ with litter size ($P = .43$) but did differ with days from parturition ($P = .004$). The difference in days from parturition resulted from the concentrations at 151 d before parturition (.732 ± .040 m*M*) being lower than those in the other periods. Like portal venous, hepatic venous concentration of esterified cholesterol (.896 ± .015 m*M*) did not differ with litter size ($P = .37$) but did differ with days from parturition ($P = .004$), and the difference was due to lower concentrations (.763 ± .031 m*M*) 151 d before parturition than during the other

**Table 1**. Means and standard errors for plasma concentrations (m*M*) of metabolites in pregnant ewes

| | Period[a] | | | | | | | Probability | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Litter size[b] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Litter (L) | Period (P) | L × P |
| Nonesterified fatty acids | | | | | | | | | | |
| Arterial | | | | | | | | .11 | .0004 | .14 |
| 0 | .176 ± .048 | .137 ± .015 | .147 ± .012 | .152 ± .008 | .185 ± .029 | .183 ± .006 | .203 ± .009 | | | |
| 1 | .133 ± .012 | .156 ± .023 | .158 ± .020 | .202 ± .021 | .206 ± .015 | .269 ± .031 | .735 ± .261 | | | |
| 2 | .116 ± .010 | .139 ± .008 | .209 ± .021 | .243 ± .022 | .296 ± .019 | .756 ± .229 | 1.162 ± .319 | | | |
| Portal venous | | | | | | | | .11 | .0004 | .19 |
| 0 | .199 ± .049 | .161 ± .020 | .164 ± .001 | .169 ± .001 | .207 ± .036 | .211 ± .014 | .225 ± .002 | | | |
| 1 | .141 ± .015 | .170 ± .025 | .177 ± .027 | .221 ± .025 | .232 ± .022 | .295 ± .026 | .787 ± .280 | | | |
| 2 | .129 ± .012 | .149 ± .011 | .239 ± .027 | .272 ± .025 | .344 ± .022 | .774 ± .234 | 1.204 ± .328 | | | |
| Hepatic venous | | | | | | | | .13 | .0005 | .22 |
| 0 | .185 ± .053 | .150 ± .017 | .141 ± .010 | .161 ± .005 | .197 ± .021 | .187 ± .009 | .198 ± .003 | | | |
| 1 | .124 ± .013 | .145 ± .023 | .148 ± .021 | .187 ± .023 | .195 ± .024 | .250 ± .038 | .757 ± .261 | | | |
| 2 | .117 ± .010 | .127 ± .008 | .202 ± .021 | .229 ± .022 | .290 ± .018 | .671 ± .212 | 1.085 ± .307 | | | |
| Glycerol | | | | | | | | | | |
| Arterial | | | | | | | | .67 | .0001 | .006 |
| 0 | .085 ± .013 | .048 ± .007 | .056 ± .008 | .056 ± .011 | .057 ± .004 | .059 ± .002 | .055 ± .003 | | | |
| 1 | .047 ± .006 | .044 ± .008 | .046 ± .008 | .055 ± .008 | .046 ± .006 | .063 ± .008 | .078 ± .010 | | | |
| 2 | .045 ± .005 | .046 ± .004 | .054 ± .005 | .058 ± .006 | .062 ± .005 | .083 ± .009 | .084 ± .006 | | | |
| Portal venous | | | | | | | | .67 | .0002 | .009 |
| 0 | .116 ± .025 | .066 ± .014 | .075 ± .019 | .079 ± .021 | .076 ± .009 | .078 ± .010 | .075 ± .004 | | | |
| 1 | .054 ± .010 | .052 ± .011 | .056 ± .013 | .066 ± .011 | .062 ± .009 | .084 ± .012 | .090 ± .010 | | | |
| 2 | .053 ± .007 | .052 ± .006 | .069 ± .007 | .070 ± .008 | .081 ± .009 | .098 ± .010 | .098 ± .006 | | | |
| Hepatic venous | | | | | | | | .0006 | .0001 | .0001 |
| 0 | .102 ± .030 | .048 ± .011 | .054 ± .019 | .060 ± .017 | .040 ± .006 | .043 ± .010 | .036 ± .009 | | | |
| 1 | .034 ± .004 | .025 ± .003 | .027 ± .004 | .032 ± .004 | .026 ± .007 | .028 ± .005 | .029 ± .004 | | | |
| 2 | .031 ± .005 | .023 ± .003 | .027 ± .003 | .028 ± .003 | .024 ± .002 | .031 ± .004 | .030 ± .003 | | | |
| Triacylglycerol | | | | | | | | | | |
| Arterial | | | | | | | | .57 | .003 | .76 |
| 0 | .071 ± .016 | .107 ± .012 | .110 ± .005 | .114 ± .014 | .102 ± .013 | .135 ± .002 | .102 ± .002 | | | |
| 1 | .068 ± .010 | .088 ± .008 | .095 ± .018 | .101 ± .016 | .108 ± .021 | .148 ± .023 | .078 ± .010 | | | |
| 2 | .079 ± .012 | .115 ± .019 | .111 ± .012 | .137 ± .017 | .175 ± .028 | .170 ± .028 | .162 ± .033 | | | |
| Portal venous | | | | | | | | .48 | .001 | .71 |
| 0 | .056 ± .007 | .094 ± .017 | .097 ± .001 | .105 ± .007 | .098 ± .014 | .124 ± .003 | .093 ± .006 | | | |
| 1 | .063 ± .009 | .082 ± .008 | .089 ± .018 | .093 ± .015 | .105 ± .022 | .140 ± .021 | .155 ± .045 | | | |
| 2 | .073 ± .011 | .110 ± .019 | .105 ± .011 | .130 ± .017 | .175 ± .029 | .166 ± .027 | .162 ± .034 | | | |
| Hepatic venous | | | | | | | | .31 | .0004 | .81 |
| 0 | .058 ± .010 | .097 ± .017 | .101 ± .001 | .106 ± .008 | .101 ± .015 | .129 ± .006 | .097 ± .003 | | | |
| 1 | .066 ± .009 | .085 ± .008 | .091 ± .017 | .098 ± .013 | .100 ± .026 | .142 ± .020 | .128 ± .036 | | | |
| 2 | .077 ± .012 | .116 ± .019 | .113 ± .012 | .136 ± .015 | .176 ± .026 | .180 ± .029 | .169 ± .032 | | | |

[a]Days before parturition Period 1 = 151 ± 1, Period 2 = 103 ± 1, Period 3 = 82 ± 1, Period 4 = 61 ± 1, Period 5 = 39 ± 1, Period 6 = 19 ± 1, and Period 7 = 6 ± 1, respectively.

[b]Litter size 0 (n = 2). Litter size 1: Period 1 through 4 (n = 6), Period 5 and 6 arterial and portal venous (n = 5) and hepatic venous (n = 6), and Period 7 arterial and portal venous (n = 6) and hepatic venous (n = 5). Litter size 2: Period 1 (n = 11), Periods 2 through 4 (n = 10), Periods 5 and 6 (n = 9), and Period 7 (n = 8).

periods. There was a net uptake of esterified cholesterol across the PDV (.950 ± .433 mmol/h; $P = .03$); however, net uptake did not differ with days from parturition ($P = .51$) or litter size ($P = .21$). Net hepatic uptake of esterified cholesterol did not differ from zero (.197 ± .349 mmol/h; $P = .57$).

### *Nonesterified Fatty Acids*

Arterial, portal venous, and hepatic venous NEFA concentrations did not differ with litter size but did differ with days from parturition (Table 1). The difference resulted from a general increase in NEFA concentration as the number of days to parturition decreased (Table 1). There was a net PDV release of NEFA (3.03 ± .36 mmol/h; $P = .0001$); however, it did not differ with litter size or days from parturition (Table 2). Net hepatic NEFA uptake differed with litter size and days from parturition (Table 2). In general, hepatic uptake increased with litter size and increased as gestation progressed (Figure 1).

### *Glycerol*

There were interactions for litter size × days from parturition for arterial, portal venous, and hepatic venous glycerol concentrations (Table 1). There was an interaction between litter size and days from parturi-

tion for net PDV glycerol release (Table 2). Net PDV release in the nonpregnant ewes decreased over time but the trend in pregnant ewes was for an increase over time (Table 2). Net hepatic glycerol uptake tended to differ with litter size and differed with days from parturition (Table 2). In general, hepatic uptake increased with litter size and increased as gestation progressed (Figure 2).

### *Triacylglycerol*

Arterial, portal venous, and hepatic venous triacylglycerol concentration did not differ with litter size but did differ with days from parturition (Table 1). This difference in triacylglycerol was due to increasing concentrations as pregnancy progressed (Table 1). There was a net PDV uptake of triacylglycerol (.67 ± .08 mmol/h; $P = .0001$). It did not differ with litter size but tended to differ with days from parturition ($P = .06$). There was a net hepatic release of triacylglycerol (.64 ± .13 mmol/h; $P = .0001$), but it did not differ with litter size or with days from parturition.

## Discussion

Studies of nutrient flux have quantified specific nutrient requirements of the gravid uterus at different

**Table 2**. Means and standard errors for net release (mmol/h) of plasma metabolites across the portal-drained viscera and liver of pregnant ewes

| | Period[a] | | | | | | | Probability | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Litter size[b] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Litter (L) | Period (P) | L × P |
| Nonesterified fatty acids | | | | | | | | | | |
| Portal-drained viscera | | | | | | | | .59 | .63 | .98 |
| 0 | 2.97 ± .35 | 2.96 ± .43 | 1.81 ± 1.24 | 1.85 ± 1.09 | 1.74 ± .67 | 3.06 ± .95 | 2.27 ± .84 | | | |
| 1 | 1.14 ± .57 | 1.88 ± .65 | 2.22 ± 1.06 | 2.29 ± .60 | 3.25 ± .97 | 3.87 ± 2.21 | 6.08 ± 2.76 | | | |
| 2 | 1.58 ± .39 | 1.14 ± .57 | 3.32 ± .80 | 3.30 ± .92 | 5.91 ± .67 | 2.63 ± 2.68 | 5.54 ± 2.69 | | | |
| Hepatic | | | | | | | | .03 | .006 | .66 |
| 0 | −1.71 ± 1.02 | −1.04 ± .32 | −2.25 ± 1.07 | −.78 ± .60 | −.61 ± 1.38 | −2.58 ± .60 | −3.03 ± .75 | | | |
| 1 | −2.41 ± .66 | −3.38 ± .46 | −3.48 ± .71 | −4.35 ± .58 | −3.50 ± .44 | −6.61 ± 2.72 | −16.54 ± 5.57 | | | |
| 2 | −1.57 ± .36 | −2.91 ± .43 | −4.28 ± .87 | −4.89 ± 1.16 | −6.63 ± .77 | −15.71 ± 3.71 | −16.69 ± 6.73 | | | |
| Splanchnic | | | | | | | | | | |
| | | | | | | | | .09 | .16 | .91 |
| 0 | 1.27 ± .67 | 1.92 ± .11 | −.44 ± 2.31 | 1.07 ± .49 | 1.14 ± .71 | .49 ± .36 | −.76 ± 1.60 | | | |
| 1 | −1.28 ± .52 | −1.49 ± .35 | −1.27 ± .75 | −2.06 ± .54 | −1.00 ± .80 | −3.91 ± .69 | −9.92 ± 4.15 | | | |
| 2 | .01 ± .58 | −1.77 ± .42 | −.96 ± .62 | −1.58 ± .80 | −.72 ± .76 | −13.07 ± 3.28 | −11.14 ± 8.77 | | | |
| Glycerol | | | | | | | | | | |
| Portal-drained viscera | | | | | | | | .50 | .09 | .04 |
| 0 | 3.93 ± .36 | 2.21 ± .71 | 1.94 ± 1.33 | 2.47 ± 1.43 | 1.42 ± .47 | 2.18 ± .94 | 2.06 ± .48 | | | |
| 1 | 1.17 ± .68 | .96 ± .37 | 1.11 ± .59 | 1.39 ± .43 | 1.93 ± .45 | 2.82 ± .62 | 1.52 ± .27 | | | |
| 2 | 1.07 ± .25 | .67 ± .26 | 1.52 ± .30 | 1.27 ± .38 | 2.25 ± .48 | 2.04 ± .60 | 1.87 ± .42 | | | |
| Hepatic | | | | | | | | .06 | .0001 | .19 |
| 0 | −1.67 ± 1.22 | −2.19 ± .22 | −2.05 ± .04 | −1.93 ± .75 | −3.14 ± .45 | −4.25 ± .08 | −4.63 ± 1.32 | | | |
| 1 | −3.06 ± 1.25 | −3.71 ± 1.42 | −3.72 ± 1.29 | −4.61 ± 1.60 | −3.74 ± .49 | −8.35 ± 1.68 | −8.29 ± 1.47 | | | |
| 2 | −3.00 ± .39 | −4.15 ± .45 | −5.09 ± .40 | −5.24 ± .64 | −7.42 ± .89 | −9.89 ± 1.01 | −10.90 ± .59 | | | |
| Splanchnic | | | | | | | | .0009 | .0001 | .19 |
| 0 | 2.56 ± .85 | .02 ± .49 | −.11 ± 1.28 | .55 ± .68 | −1.72 ± .02 | −2.07 ± 1.02 | −2.58 ± 1.80 | | | |
| 1 | −1.89 ± .71 | −2.75 ± 1.05 | −2.61 ± .71 | −3.22 ± 1.20 | −2.14 ± .44 | −5.29 ± 1.01 | −6.72 ± 1.31 | | | |
| 2 | −1.93 ± .37 | −3.49 ± .35 | −3.58 ± .40 | −3.97 ± .37 | −5.17 ± .57 | −7.84 ± .64 | −9.03 ± .81 | | | |
| Triacylglycerol | | | | | | | | | | |
| Portal-drained viscera | | | | | | | | .11 | .06 | .91 |
| 0 | −2.08 ± 1.36 | −1.62 ± .40 | −1.25 ± .60 | −1.05 ± .90 | −.26 ± .11 | −1.17 ± .21 | −.87 ± .29 | | | |
| 1 | −.66 ± .21 | −.80 ± .22 | −.72 ± .19 | −.91 ± .25 | −.38 ± .13 | −1.11 ± .53 | −.61 ± .39 | | | |
| 2 | −.78 ± .23 | −.73 ± .25 | −.77 ± .33 | −.79 ± .18 | .02 ± .26 | −.39 ± .53 | −.03 ± .19 | | | |
| Hepatic | | | | | | | | .59 | .67 | .9998 |
| 0 | .17 ± .46 | .20 ± .11 | .17 ± .17 | −.002 ± .06 | .23 ± .17 | .37 ± .40 | .32 ± .18 | | | |
| 1 | .36 ± .29 | .41 ± .56 | .23 ± .52 | .48 ± .41 | −.13 ± .55 | 1.73 ± .89 | 1.14 ± .58 | | | |
| 2 | .39 ± .10 | .71 ± .26 | .90 ± .39 | .57 ± .35 | .11 ± .81 | 1.66 ± .96 | 1.14 ± .65 | | | |
| Splanchnic | | | | | | | | .09 | .07 | .99 |
| 0 | −1.91 ± .90 | −1.42 ± .51 | −1.09 ± .78 | −1.05 ± .84 | −.03 ± .28 | −.80 ± .62 | −.55 ± .11 | | | |
| 1 | −.30 ± .17 | −.39 ± .49 | −.49 ± .41 | −.43 ± .59 | −.43 ± .51 | .93 ± 1.10 | .83 ± .45 | | | |
| 2 | −.39 ± .24 | −.02 ± .33 | .13 ± .23 | −.22 ± .42 | .13 ± .68 | 1.27 ± .69 | 1.11 ± .68 | | | |

[a]Days before parturition Period 1 = 151 ± 1, Period 2 = 103 ± 1, Period 3 = 82 ± 1, Period 4 = 61 ± 1, Period 5 = 39 ± 1, Period 6 = 19 ± 1, and Period 7 = 6 ± 1, respectively.

[b]Litter size 0 (n = 2). Litter size 1: Period 1 through 4 (n = 6), Period 5 and 6 arterial and portal venous (n = 5) and hepatic venous (n = 6), and Period 7 arterial and portal venous (n = 6) and hepatic venous (n = 5). Litter size 2: Period 1 (n = 11), Periods 2 through 4 (n = 10), Periods 5 and 6 (n = 9), and Period 7 (n = 8).

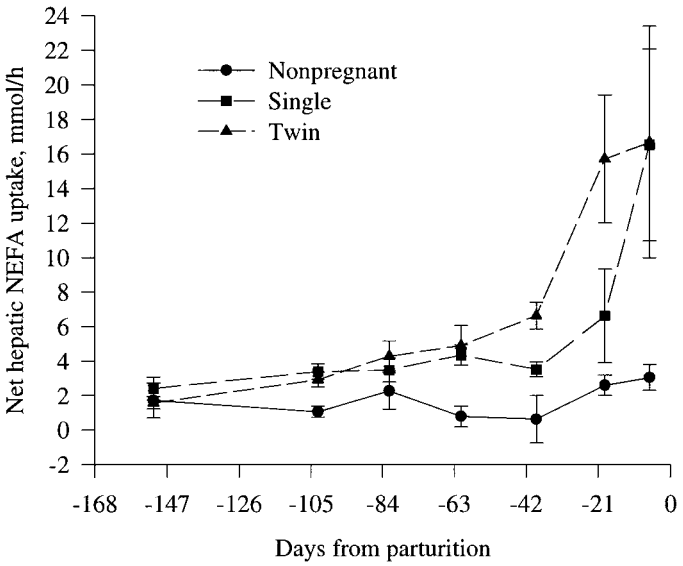


**Figure 1**. Means and standard errors for net hepatic NEFA uptake of the ewe. Nonpregnant (n = 2). Single: –151 through –61 d (n = 6), –39 and –19 d (n = 4), and –6 d (n = 5). Twins: –151 d (n = 11), –103 through –61 d (n = 10), –39 and –19 d (n = 9), and –6 d (n = 8).

stages of pregnancy (Battaglia and Meschia, 1981; Bell et al., 1986). The nutrient demands of the gravid uterus suggest that the number of fetuses and stage of pregnancy will affect the manner in which nutrients are used by the dam in support of the pregnancy. Freetly and Ferrell (1998) demonstrated that increases in net maternal hepatic glucose release were similar to the

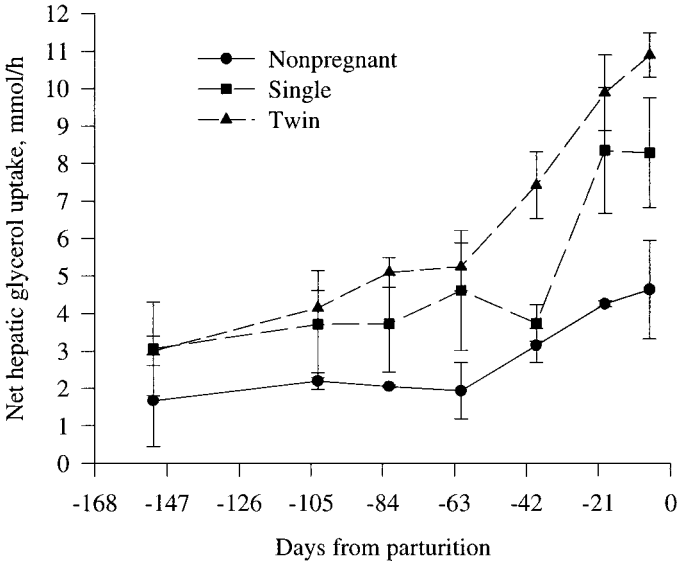


**Figure 2**. Means and standard errors for net hepatic glycerol uptake of the ewe. Nonpregnant (n = 2). Single: –151 through –61 d (n = 6), –39 and –19 d (n = 4), and –6 d (n = 5). Twins: –151 d (n = 11), –103 through –61 d (n = 10), –39 and –19 d (n = 9), and –6 d (n = 8).

expected increases in glucose uptake by the gravid uterus. Although shifts in the pattern of hepatic substrate uptake were also shown, an insufficient amount of carbon had been accounted for to support the synthesis of the metabolites released by the liver.

A potential source of carbon for hepatic metabolism is esterified cholesterol. Noble et al. (1975) reported that the majority of cholesterol esters are synthesized in the plasma of sheep. In the current study, there was a net uptake of esterified cholesterol by the PDV, a result that is consistent with the observations of Bergman et al. (1971), who reported a net PDV uptake of esterified cholesterol in nonpregnant, nonlactating ewes. Although Bergman et al. (1971) reported a net hepatic uptake of esterified cholesterol, this study found that net flux did not differ from 0. The difference between the two studies may result from differences in methodology. The measurements of Bergman et al. (1971) were based on uptake of radiolabeled esterified cholesterol, compared to the net flux measurement used in this study. Noble et al. (1975) reported synthesis of cholesterol esters in the liver of sheep, suggesting that the flux between the liver and blood may be bidirectional. This study does not support the hypothesis that esterified cholesterol is a net contributor to hepatic carbon balance.

A primary adaptation to pregnancy in the ewe is for an increased entry of NEFA into the plasma (Pethick et al., 1983). Petterson et al. (1993) concluded that the increase in circulating NEFA concentration during pregnancy was independent of energy balance. Pethick et al. (1983) reported that NEFA entry rates are linearly related to arterial concentration. Using the linear relationship described by Pethick et al. (1983) and our arterial NEFA concentrations, we would predict that NEFA entry rates increased as pregnancy progressed (Figure 3). In the current study, feed intakes were lower in late gestation. We suggest that the majority of the increased NEFA entry probably resulted from increased lipolysis in adipose tissue. Most dietary lipids are absorbed via the lymphatic system and released into circulation at the subclavian vein; therefore, net PDV release is more indicative of lipid metabolism of the mesenteric fat than it is of dietary lipid. Net PDV release of NEFA did not differ with litter size or days before parturition, but its relative contribution to whole-animal entry rate decreased as pregnancy progressed (Figure 3). Before breeding, the net PDV flux was equal to 27% of the estimated whole-animal NEFA entry rate, but it decreased to 12% in ewes with twins 19 d before parturition.

Both radiolabeled isotope (Pethick et al., 1983) and arterial-venous difference (James et al., 1971) studies have concluded that the gravid uterus does not utilize NEFA. Their findings suggested that the increase in NEFA entry rate was used primarily by maternal tissues. In the current study, the hepatic uptake of NEFA by the maternal liver accounted for 32% of the entry rate before ewes were bred and 78% of the entry rate

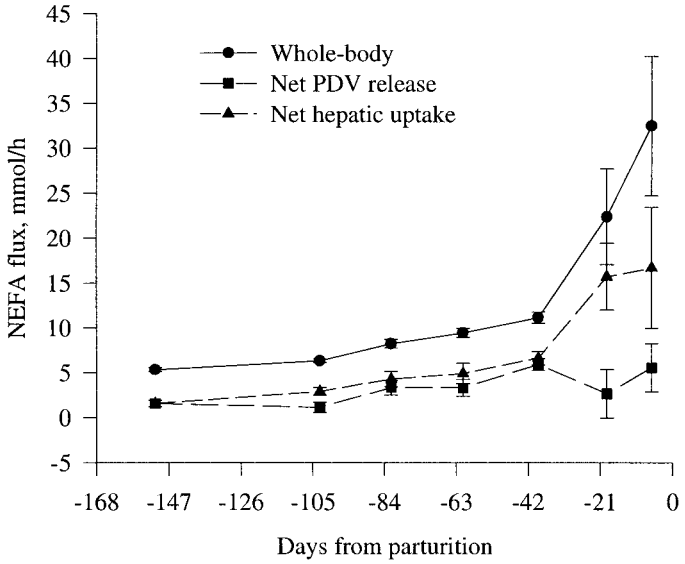


**Figure 3**. Means and standard errors for estimated NEFA turnover rate and net portal-drained viscera (PDV) NEFA release and net hepatic NEFA uptake of ewes pregnant with twins: –151 d (n = 11), –103 through –61 d (n = 10), –39 and –19 d (n = 9), and –6 d (n = 8).

in ewes with twins 19 d before parturition (Figure 3). Our finding that hepatic uptake increased with stage of pregnancy differs from the observations of Katz and Bergman (1969a), who determined that net hepatic NEFA uptake did not differ with pregnancy in fed sheep. The two studies differ in that the estimates of hepatic NEFA uptake were lower for nonpregnant and early-pregnant ewes in this study than in that of Katz and Bergman (1969a). Bergman et al. (1971) determined that hepatic NEFA removal (24%) and hind-half removal (26%) were similar in the mature, nonpregnant, nonlactating ewe. Pethick et al. (1983) estimated that the relative contribution of NEFA as a source of respiratory carbon dioxide in muscle varied with physiological state, with 7% of the carbon dioxide originating from NEFA in fed, nonpregnant ewes and 42% originating from NEFA in feed-restricted, pregnant ewes. In the current study, the difference between NEFA entry rate and hepatic uptake did not change with litter size or stage of pregnancy (5.4 mmol/h), suggesting that NEFA usage by peripheral tissues remains relatively constant and that the liver responds to increased NEFA entry during pregnancy by increasing NEFA uptake (Figure 3).

Bergman et al. (1968) reported that glycerol turnover rate was linearly related to glycerol concentration and was described by the equation $f(x) = .125 + .00524x$, where $f(x)$ is expressed in mmol/h·kg$^{.75}$ of BW and x is expressed as μmol/L. Based on the findings of Bergman et al. (1968), we estimate that twin-bearing ewes 19 d before parturition in this study had a whole-body glycerol turnover rate of 16.8 mmol/h. Net PDV glycerol release in these same ewes was 2.0 mmol/h, which accounts for 12% of the entry rate. Glycerol release from the PDV most likely is a combination of glycerol from dietary origin and glycerol released from the mesenteric fat. These same ewes had a hepatic uptake of 9.9 mmol/h, which accounted for 59% of the whole-body glycerol uptake. The net uptake of glycerol by the gravid uterus is low relative to net hepatic uptake. Assuming the gravid uterus weighs 4.84 kg 19 d before parturition (Freetly and Ferrell, 1997) and the uterine blood flow rate is 239 mL/(kg·min) (Caton et al., 1979), we calculate a blood flow of 69 L/h. James et al. (1971) reported an arterial-venous difference across the gravid uterus of .002 mmol/L. Based on the calculated blood flow rate and the reported arterial-venous difference, we estimate an uptake rate of .14 mmol/h by the gravid uterus, compared to 8.3 mmol/h by the liver, in ewes with singles 19 d before parturition.

As pregnancy progresses in the ewe, there is an increased demand on the maternal liver for the synthesis of glucose and urea. As has previously been shown (Freetly and Ferrell, 1998), part of this increased demand for substrates is met by shifts in lactate metabolism. Glycerol can also be used as a precursor for gluconeogenesis. Bergman (1968) reported that 28% of the glucose originated from glycerol in pregnant, hypoglycemic ewes but 4.7% of the glucose originated from glycerol in fed, nonpregnant ewes.

In the current study there was a net uptake of triacylglycerol by the PDV. This net uptake is consistent with the radiotracer study of Bergman et al. (1971), who found an uptake of triacylglycerol across the PDV. This net uptake of triacylglycerol probably represents uptake by the mesenteric adipose tissue. In this study, there was a net release of triacylglycerol across the liver. The radiotracer data of Bergman et al. (1971) indicate that there is an uptake of triacylglycerol by the sheep liver. Net flux data are inconsistent on whether triacylglycerol is taken up or released by the liver. Kerr et al. (1988) reported a net uptake of triacylglycerol in nonpregnant, nonlactating ewes that were fed at maintenance, whereas the rate of uptake increased when feed was withheld from the ewes. Reid et al. (1979) reported that in lactating cows there was a net release of triacylglycerol; however, when cows were unfed, there was an uptake of triacylglycerol. These studies suggest that triacylglycerol flux across the liver is dependent on nutrient status of the animal. In the current study, ewes were either in zero or positive weight balance, suggesting a net release of triacylglycerol from the liver.

In the current study, carbon taken up by the liver accounted for 79% of the carbon released by the liver in the nonpregnant ewe (Figure 4). In the pregnant ewe 19 d before parturition, 75% of the carbon was accounted for in ewes with singles and 93% in ewes with twins (Figure 4). Ketone body fluxes were not measured in this study but were estimated based on the findings of Katz and Bergman (1969a). The liver respiration quotient (.70) was set similarly to that reported

in lactating cows (Reynolds et al., 1988). Pethick et al. (1983) reported the composition of NEFA in the arterial blood of pregnant ewes, and based on the relative proportions of each NEFA, the average number of carbons for NEFA and esterified fatty acids in triacylglycerol was set to 17.6. The average carbon number (4.3) for amino acids was calculated in a manner similar to that for NEFA and was based on the net amino acid uptakes reported by Wolff et al. (1972).

Glucose release by the liver accounted for the majority of the carbon released by the liver in both the nonpregnant ewe (37%) and the ewe pregnant with twins (42%; Figure 4). Carbon dioxide was the second largest form in which carbon was released from the liver, it accounted for 30% in nonpregnant ewes and 27% in ewes pregnant with twins (Figure 4). In the nonpregnant ewe, amino acids (39%) and propionate (34%) represented the primary sources of carbon uptake (Figure 4). In the pregnant ewe, the net uptake of propionate by liver was greater, but it was a smaller fraction of the carbon uptake (21%; Figure 4). In ewes pregnant with twins, the majority of the carbon taken up by the liver was in the form of NEFA (38%), compared to 8% in nonpregnant ewes (Figure 4). In late pregnancy, lipogenesis decreases and lipolysis increases in adipose tissue (Vernon et al., 1981; Guesnet et al., 1991). Our data suggest that the maternal liver mediates intermediary metabolism by using the available metabolites from the diet and from peripheral tissues to meet the nutrient demands of the conceptus. In conclusion, these data demonstrate NEFA are an important metabolite when determining the carbon balance across the liver, and their relative contribution to the carbon balance increases as pregnancy progresses. Whereas the relative proportion of unaccounted carbon decreases in the pregnant ewe, the absolute amount of unaccounted carbon between the nonpregnant and late-pregnant ewe with twins is similar (Figure 4). This observation suggests that the unaccounted carbon is a part of a process that is unresponsive to increases in whole-animal energy metabolism. These processes may include metabolism of steroids or other metabolites in which the liver is the primary site of catabolism.

## Implications

The amount of each substrate used by the liver during pregnancy depends on the stage of gestation and the number of fetuses. As gestation progresses and nutrient demand increases, lipids become important sources of energy for the liver. The liver responds to an increased availability of lipids, as the result of increased release from adipose tissue. The increased availability of lipids allows the liver to spare other metabolites, including propionate and amino acids, from oxidation, allowing them to be used for alternative purposes such as the synthesis of glucose. These data suggest that ewes need to have an adequate amount of body lipid in late preg-

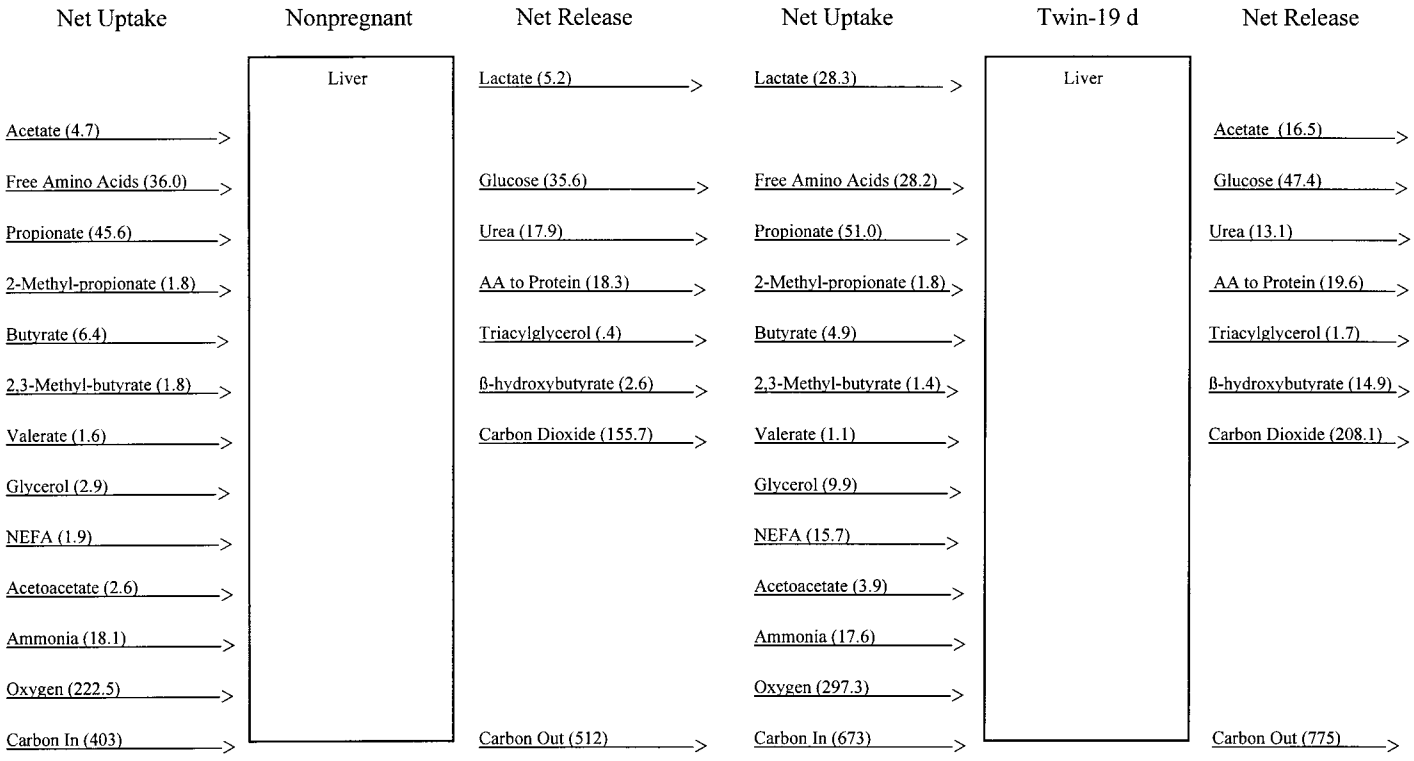


**Figure 4**. Net metabolite flux (mmol/h) across the liver of nonpregnant and ewes pregnant with twins 19 d before parturition. Flux estimated based on presented data and from Freetly and Ferrell, 1997, 1998; Katz and Bergman, 1969a; Pethick et al., 1983; and Wolff et al., 1972.

nancy to allow for this increased supply of lipid to the liver.

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